

WHAT IS CLAIMED IS:

1. A method for at least one of the following:

- (A) identifying a mutation point of a gene derived from a mutant of a coryneform bacterium,
- (B) measuring an expression amount of a gene derived from a coryneform bacterium,
- (C) analyzing an expression profile of a gene derived from a coryneform bacterium,
- (D) analyzing expression patterns of genes derived from a coryneform bacterium, or
- (E) identifying a gene homologous to a gene derived from a coryneform bacterium,

said method comprising:

- (a) producing a polynucleotide array by adhering to a solid support at least two polynucleotides selected from the group consisting of first polynucleotides comprising the nucleotide sequence represented by any one of SEQ ID NOS:1 to 3501, second polynucleotides which hybridize with the first polynucleotides under stringent conditions, and third polynucleotides comprising a sequence of 10 to 200 continuous bases of the first or second polynucleotides,
- (b) incubating the polynucleotide array with at least one of a labeled polynucleotide derived from a coryneform bacterium, a labeled polynucleotide derived from a mutant

of the coryneform bacterium or a labeled polynucleotide to be examined, under hybridization conditions,

- (c) detecting any hybridization, and
- (d) analyzing the result of the hybridization.

2. The method according to claim 1, wherein the coryneform bacterium is a microorganism belonging to the genus *Corynebacterium*, the genus *Brevibacterium*, or the genus *Microbacterium*.

3. The method according to claim 2, wherein the microorganism belonging to the genus *Corynebacterium* is selected from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium acetoacidophilum*, *Corynebacterium acetoglutamicum*, *Corynebacterium callunae*, *Corynebacterium herculis*, *Corynebacterium lilium*, *Corynebacterium melassecola*, *Corynebacterium thermoaminogenes*, and *Corynebacterium ammoniagenes*.

4. The method according to claim 1, wherein the polynucleotide derived from a coryneform bacterium, the polynucleotide derived from a mutant of the coryneform bacterium or the polynucleotide to be examined is a gene relating to the biosynthesis of at least one compound selected from an amino acid, a nucleic acid, a vitamin, a saccharide, an organic acid, and analogues thereof.

5. The method according to claim 1, wherein the polynucleotide to be examined is derived from *Escherichia coli*.

6. A polynucleotide array, comprising:

at least two polynucleotides selected from the group consisting of first polynucleotides comprising the nucleotide sequence represented by any one of SEQ ID NOS:1 to 3501, second polynucleotides which hybridize with the first polynucleotides under stringent conditions, and third polynucleotides comprising 10 to 200 continuous bases of the first or second polynucleotides, and

a solid support adhered thereto.

7. A polynucleotide comprising the nucleotide sequence represented by SEQ ID NO:1 or a polynucleotide having a homology of at least 80% with the polynucleotide.

8. A polynucleotide comprising any one of the nucleotide sequences represented by SEQ ID NOS:2 to 3431, or a polynucleotide which hybridizes with the polynucleotide under stringent conditions.

9. A polynucleotide encoding a polypeptide having any one of the amino acid sequences represented by SEQ ID

NOS:3502 to 6931, or a polynucleotide which hybridizes therewith under stringent conditions.

10. A polynucleotide which is present in the 5' upstream or 3' downstream of a polynucleotide comprising the nucleotide sequence of any one of SEQ ID NOS:2 to 3431 in a whole polynucleotide comprising the nucleotide sequence represented by SEQ ID NO:1, and has an activity of regulating an expression of the polynucleotide.

11. A polynucleotide comprising 10 to 200 continuous bases in the nucleotide sequence of the polynucleotide of any one of claims 7 to 10, or a polynucleotide comprising a nucleotide sequence complementary to the polynucleotide comprising 10 to 200 continuous based.

12. A recombinant DNA comprising the polynucleotide of any one of claims 8 to 11.

13. A transformant comprising the polynucleotide of any one of claims 8 to 11 or the recombinant DNA of claim 12.

14. A method for producing a polypeptide, comprising:

culturing the transformant of claim 13 in a medium to produce and accumulate a polypeptide encoded by the polynucleotide of claim 8 or 9 in the medium, and recovering the polypeptide from the medium.

15. A method for producing at least one of an amino acid, a nucleic acid, a vitamin, a saccharide, an organic acid, and analogues thereof, comprising:

culturing the transformant of claim 13 in a medium to produce and accumulate at least one of an amino acid, a nucleic acid, a vitamin, a saccharide, an organic acid, and analogues thereof in the medium, and

recovering the at least one of the amino acid, the nucleic acid, the vitamin, the saccharide, the organic acid, and analogues thereof from the medium.

16. A polypeptide encoded by a polynucleotide comprising the nucleotide sequence selected from SEQ ID NOS:2 to 3431.

17. A polypeptide comprising the amino acid sequence selected from SEQ ID NOS:3502 to 6931.

18. The polypeptide according to claim 16 or 17, wherein at least one amino acid is deleted, replaced, inserted or added, said polypeptides having an activity

which is substantially the same as that of the polypeptide without said at least one amino acid deletion, replacement, insertion or addition.

19. A polypeptide comprising an amino acid sequence having a homology of at least 60% with the amino acid sequence of the polypeptide of claim 16 or 17, and having an activity which is substantially the same as that of the polypeptide.

20. An antibody which recognizes the polypeptide of any one of claims 16 to 19.

21. A polypeptide array, comprising:

at least one polypeptide or partial fragment polypeptide selected from the polypeptides of claims 16 to 19 and partial fragment polypeptides of the polypeptides, and

a solid support adhered thereto.

22. A polypeptide array, comprising:

at least one antibody which recognizes a polypeptide or partial fragment polypeptide selected from the polypeptides of claims 16 to 19 and partial fragment polypeptides of the polypeptides, and

a solid support adhered thereto.

23. A system based on a computer for identifying a target sequence or a target structure motif derived from a coryneform bacterium, comprising the following:

- (i) a user input device that inputs at least one nucleotide sequence information selected from SEQ ID NOS:1 to 3501, and target sequence or target structure motif information;
- (ii) a data storage device for at least temporarily storing the input information;
- (iii) a comparator that compares the at least one nucleotide sequence information selected from SEQ ID NOS:1 to 3501 with the target sequence or target structure motif information, recorded by the data storage device for screening and analyzing nucleotide sequence information which is coincident with or analogous to the target sequence or target structure motif information; and
- (iv) an output device that shows a screening or analyzing result obtained by the comparator.

24. A method based on a computer for identifying a target sequence or a target structure motif derived from a coryneform bacterium, comprising the following:

- (i) inputting at least one nucleotide sequence information selected from SEQ ID NOS:1 to 3501, target

sequence information or target structure motif information into a user input device;

- (ii) at least temporarily storing said information;
- (iii) comparing the at least one nucleotide sequence information selected from SEQ ID NOS:1 to 3501 with the target sequence or target structure motif information; and
- (iv) screening and analyzing nucleotide sequence information which is coincident with or analogous to the target sequence or target structure motif information.

25. A system based on a computer for identifying a target sequence or a target structure motif derived from a coryneform bacterium, comprising the following:

- (i) a user input device that inputs at least one amino acid sequence information selected from SEQ ID NOS:3502 to 7001, and target sequence or target structure motif information;
- (ii) a data storage device for at least temporarily storing the input information;
- (iii) a comparator that compares the at least one amino acid sequence information selected from SEQ ID NOS:3502 to 7001 with the target sequence or target structure motif information, recorded by the data storage device for screening and analyzing amino acid sequence information which is coincident with or analogous to the target sequence or target structure motif information; and



(iv) an output device that shows a screening or analyzing result obtained by the comparator.

26. A method based on a computer for identifying a target sequence or a target structure motif derived from a coryneform bacterium, comprising the following:

- (i) inputting at least one amino acid sequence information selected from SEQ ID NOS:3502 to 7001, and target sequence information or target structure motif information into a user input device;
- (ii) at least temporarily storing said information;
- (iii) comparing the at least one amino acid sequence information selected from SEQ ID NOS:3502 to 7001 with the target sequence or target structure motif information; and
- (iv) screening and analyzing amino acid sequence information which is coincident with or analogous to the target sequence or target structure motif information.

27. A system based on a computer for determining a function of a polypeptide encoded by a polynucleotide having a target nucleotide sequence derived from a coryneform bacterium, comprising the following:

- (i) a user input device that inputs at least one nucleotide sequence information selected from SEQ ID NOS:2 to 3501, function information of a polypeptide encoded by

the nucleotide sequence, and target nucleotide sequence information;

(ii) a data storage device for at least temporarily storing the input information;

(iii) a comparator that compares the at least one nucleotide sequence information selected from SEQ ID NOS:2 to 3501 with the target nucleotide sequence information for determining a function of a polypeptide encoded by a polynucleotide having the target nucleotide sequence which is coincident with or analogous to the polynucleotide having at least one nucleotide sequence selected from SEQ ID NOS:2 to 3501; and

(iv) an output devices that shows a function obtained by the comparator.

28. A method based on a computer for determining a function of a polypeptide encoded by a polypeptide encoded by a polynucleotide having a target nucleotide sequence derived from a coryneform bacterium, comprising the following:

(i) inputting at least one nucleotide sequence information selected from SEQ ID NOS:2 to 3501, function information of a polypeptide encoded by the nucleotide sequence, and target nucleotide sequence information;

(ii) at least temporarily storing said information;

- (iii) comparing the at least one nucleotide sequence information selected from SEQ ID NOS:2 to 3501 with the target nucleotide sequence information; and
- (iv) determining a function of a polypeptide encoded by a polynucleotide having the target nucleotide sequence which is coincident with or analogous to the polynucleotide having at least one nucleotide sequence selected from SEQ ID NOS:2 to 3501.

29. A system based on a computer for determining a function of a polypeptide having a target amino acid sequence derived from a coryneform bacterium, comprising the following:

- (i) a user input device that inputs at least one amino acid sequence information selected from SEQ ID NOS:3502 to 7001, function information based on the amino acid sequence, and target amino acid sequence information;
- (ii) a data storing device for at least temporarily storing the input information;
- (iii) a comparator that compares the at least one amino acid sequence information selected from SEQ ID NOS:3502 to 7001 with the target amino acid sequence information for determining a function of a polypeptide having the target amino acid sequence which is coincident with or analogous to the polypeptide having at least one amino acid sequence selected from SEQ ID NOS:3502 to 7001; and

(iv) an output device that shows a function obtained by the comparator.

30. A method based on a computer for determining a function of a polypeptide having a target amino acid sequence derived from a coryneform bacterium, comprising the following:

(i) inputting at least one amino acid sequence information selected from SEQ ID NOS:3502 to 7001, function information based on the amino acid sequence, and target amino acid sequence information;

(ii) at least temporarily storing said information;

(iii) comparing the at least one amino acid sequence information selected from SEQ ID NOS:3502 to 7001 with the target amino acid sequence information; and

(iv) determining a function of a polypeptide having the target amino acid sequence which is coincident with or analogous to the polypeptide having at least one amino acid sequence selected from SEQ ID NOS:3502 to 7001.

31. The system according to any one of claims 23, 25, 27 and 29, wherein a coryneform bacterium is a microorganism of the genus *Corynebacterium*, the genus *Brevibacterium*, or the genus *Microbacterium*.

32. The method according to any one of claims 24, 26, 28 and 30, wherein a coryneform bacterium is a microorganism of the genus *Corynebacterium*, the genus *Brevibacterium*, or the genus *Microbacterium*.

33. The system according to claim 31, wherein the microorganism belonging to the genus *Corynebacterium* is selected from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium acetoacidophilum*, *Corynebacterium acetoglutamicum*, *Corynebacterium callunae*, *Corynebacterium herculis*, *Corynebacterium lilium*, *Corynebacterium melassecola*, *Corynebacterium thermoaminogenes*, and *Corynebacterium ammoniagenes*.

34. The method according to claim 32, wherein the microorganism belonging to the genus *Corynebacterium* is selected from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium acetoacidophilum*, *Corynebacterium acetoglutamicum*, *Corynebacterium callunae*, *Corynebacterium herculis*, *Corynebacterium lilium*,

*Corynebacterium melassecola*, *Corynebacterium thermoaminogenes*, and *Corynebacterium ammoniagenes*.

35. A recording medium or storage device which is readable by a computer in which at least one nucleotide sequence information selected from SEQ ID NOS:1 to 3501 or function information based on the nucleotide sequence is recorded, and is usable in the system of claim 23 or 27 or the method of claim 24 or 28.

36. A recording medium or storage device which is readable by a computer in which at least one amino acid sequence information selected from SEQ ID NOS:3502 to 7001 or function information based on the amino acid sequence is recorded, and is usable in the system of claim 25 or 29 or the method of claim 26 or 30.

37. The recording medium or storage device according to claim 35 or 36, which is a computer readable recording medium selected from the group consisting of a floppy disc, a hard disc, a magnetic tape, a random access memory (RAM), a read only memory (ROM), a magneto-optic disc (MO), CD-ROM, CD-R, CD-RW, DVD-ROM, DVD-RAM and DVD-RW.

38. A polypeptide having a homoserine dehydrogenase activity, comprising an amino acid sequence

in which the Val residue at the 59th in the amino acid sequence of homoserine dehydrogenase derived from a coryneform bacterium is replaced with an amino acid residue other than a Val residue.

39. A polypeptide comprising an amino acid sequence in which the Val residue at the 59th position in the amino acid sequence as represented by SEQ ID NO:6952 is replaced with an amino acid residue other than a Val residue.

40. The polypeptide according to claim 38 or 39, wherein the Val residue at the 59th position is replaced with an Ala residue.

41. A polypeptide having pyruvate carboxylase activity, comprising an amino acid sequence in which the Pro residue at the 458th position in the amino acid sequence of pyruvate carboxylase derived from a coryneform bacterium is replaced with an amino acid residue other than a Pro residue.

42. A polypeptide comprising an amino acid sequence in which the Pro residue at the 458th position in the amino acid sequence represented by SEQ ID NO:4265 is

replaced with an amino acid residue other than a Pro residue.

43. The polypeptide according to claim 41 or 42, wherein the Pro residue at the 458th position is replaced with a Ser residue.

44. The polypeptide according to any one of claims 38 to 43, which is derived from *Corynebacterium glutamicum*.

45. A DNA encoding the polypeptide of any one of claims 38 to 44.

46. A recombinant DNA comprising the DNA of claim 45.

47. A transformant comprising the recombinant DNA of claim 46.

48. A transformant comprising in its chromosome the DNA of claim 45.

49. The transformant according to claim 47 or 48, which is derived from a coryneform bacterium.



50. The transformant according to claim 49, which is derived from *Corynebacterium glutamicum*.

51. A method for producing L-lysine, comprising:  
culturing the transformant of any one of claims 47 to 50 in a medium to produce and accumulate L-lysine in the medium, and  
recovering the L-lysine from the culture.

52. A method for breeding a coryneform bacterium using the nucleotide sequence information represented by SEQ ID NOS:1 to 3431, comprising the following:

- (i) comparing a nucleotide sequence of a genome or gene of a production strain derived a coryneform bacterium which has been subjected to mutation breeding so as to produce at least one compound selected from an amino acid, a nucleic acid, a vitamin, a saccharide, an organic acid, and analogous thereof by a fermentation method, with a corresponding nucleotide sequence in SEQ ID NOS:1 to 3431;
- (ii) identifying a mutation point present in the production strain based on a result obtained by (i);
- (iii) introducing the mutation point into a coryneform bacterium which is free of the mutation point, or deleting the mutation point from a coryneform bacterium having the mutation point; and

(iv) examining productivity by the fermentation method of the compound selected in (i) of the coryneform bacterium obtained in (iii).

53. The method according to claim 52, wherein the gene is a gene encoding an enzyme in a biosynthetic pathway or a signal transmission pathway.

54. The method according to claim 52, wherein the mutation point is a mutation point relating to a useful mutation which improves or stabilizes the productivity.

55. A method for breeding a coryneform bacterium using the nucleotide sequence information represented by SEQ ID NOS:1 to 3431, comprising:

- (i) comparing a nucleotide sequence of a genome or gene of a production strain derived a coryneform bacterium which has been subjected to mutation breeding so as to produce at least one compound selected from an amino acid, a nucleic acid, a vitamin, a saccharide, an organic acid, and analogous thereof by a fermentation method, with a corresponding nucleotide sequence in SEQ ID NOS:1 to 3431;
- (ii) identifying a mutation point present in the production strain based on a result obtain by (i);
- (iii) deleting a mutation point from a coryneform bacterium having the mutation point; and

(iv) examining productivity by the fermentation method of the compound selected in (i) of the coryneform bacterium obtained in (iii).

56. The method according to claim 55, wherein the gene is a gene encoding an enzyme in a biosynthetic pathway or a signal transmission pathway.

57. The method according to claim 55, wherein the mutation point is a mutation point which decreases or destabilizes the productivity.

58. A method for breeding a coryneform bacterium using the nucleotide sequence information represented by SEQ ID NOS:2 to 3431, comprising the following:

- (i) identifying an isozyme relating to biosynthesis of at least one compound selected from an amino acid, a nucleic acid, a vitamin, a saccharide, an organic acid, and analogous thereof, based on the nucleotide sequence information represented by SEQ ID NOS:2 to 3431;
- (ii) classifying the isozyme identified in (i) into an isozyme having the same activity;
- (iii) mutating all genes encoding the isozyme having the same activity simultaneously; and

(iv) examining productivity by a fermentation method of the compound selected in (i) of the coryneform bacterium which have been transformed with the gene obtained in (iii).

59. A method for breeding a coryneform bacterium using the nucleotide sequence information represented by SEQ ID NOS:2 to 3431, comprising the following:

- (i) arranging a function information of an open reading frame (ORF) represented by SEQ ID NOS:2 to 3431;
- (ii) allowing the arranged ORF to correspond to an enzyme on a known biosynthesis or signal transmission pathway;
- (iii) explicating an unknown biosynthesis pathway or signal transmission pathway of a coryneform bacterium in combination with information relating known biosynthesis pathway or signal transmission pathway of a coryneform bacterium;
- (iv) comparing the pathway explicated in (iii) with a biosynthesis pathway of a target useful product; and
- (v) transgenetically varying a coryneform bacterium based on the nucleotide sequence information to either strengthen a pathway which is judged to be important in the biosynthesis of the target useful product in (iv) or weaken a pathway which is judged not to be important in the biosynthesis of the target useful product in (iv).

60. A coryneform bacterium, bred by the method of any one of claims 52 to 59.

61. The coryneform bacterium according to claim 60, which is a microorganism belonging to the genus *Corynebacterium*, the genus *Brevibacterium*, or the genus *Microbacterium*.

62. The coryneform bacterium according to claim 61, wherein the microorganism belonging to the genus *Corynebacterium* is selected from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium acetoacidophilum*, *Corynebacterium acetoglutamicum*, *Corynebacterium callunae*, *Corynebacterium herculis*, *Corynebacterium lilium*, *Corynebacterium melassecola*, *Corynebacterium thermoaminogenes*, and *Corynebacterium ammoniagenes*.

63. A method for producing at least one compound selected from an amino acid, a nucleic acid, a vitamin, a saccharide, an organic acid and an analogue thereof, comprising:

culturing a coryneform bacterium of any one of claims 60 to 62 in a medium to produce and accumulate at least one compound selected from an amino acid, a nucleic

acid, a vitamin, a saccharide, an organic acid, and analogues thereof;

recovering the compound from the culture.

64. The method according to claim 63, wherein the compound is L-lysine.

65. A method for identifying a protein relating to useful mutation based on proteome analysis, comprising the following:

(i) preparing

a protein derived from a bacterium of a production strain of a coryneform bacterium which has been subjected to mutation breeding by a fermentation process so as to produce at least one compound selected from an amino acid, a nucleic acid, a vitamin, a saccharide, an organic acid, and analogues thereof, and

a protein derived from a bacterium of a parent strain of the production strain;

(ii) separating the proteins prepared in (i) by two dimensional electrophoresis;

(iii) detecting the separated proteins, and comparing an expression amount of the protein derived from the production strain with that derived from the parent strain;

(iv) treating the protein showing different expression amounts as a result of the comparison with a peptidase to extract peptide fragments;

(v) analyzing amino acid sequences of the peptide fragments obtained in (iv); and

(vi) comparing the amino acid sequences obtained in (v) with the amino acid sequence represented by SEQ ID NOS:3502 to 7001 to identifying the protein having the amino acid sequences.

66. The method according to claim 65, wherein the coryneform bacterium is a microorganism belonging to the genus *Corynebacterium*, the genus *Brevibacterium*, or the genus *Microbacterium*.

67. The method according to claim 66, wherein the microorganism belonging to the genus *Corynebacterium* is selected from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium acetoacidophilum*, *Corynebacterium acetoglutamicum*, *Corynebacterium callunae*, *Corynebacterium herculis*, *Corynebacterium lilium*, *Corynebacterium melassecola*, *Corynebacterium thermoaminogenes*, and *Corynebacterium ammoniagenes*.

68. A biologically pure culture of *Corynebacterium glutamicum* AHP-3 (FERM BP-7382).